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Short communication

Would CLSI M53-A have helped in the diagnosis of HIV in Canada? Results of the performance of Canadian laboratories participating in a recent NLHRS proficiency testing panel containing HIV-1 antigen positive (antibody negative) and HIV-2 samples

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ABSTRACT

Introduction: The Clinical and Laboratory Standards Institute recently published M53-A, *Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus (HIV) Infection; Approved Guideline* (2011), which includes a state of the art algorithm for identifying HIV-1 acute and HIV-2 infections. To assess the ability of Canadian laboratories to detect these sample types and the impact of M53-A, the National Laboratory for HIV Reference Services distributed a special proficiency testing panel.
Methods: HIVS425-2012Nov22 was sent to 42 laboratories across Canada. It contained one HIV negative sample (B), two HIV-1 positive samples (A and E), one HIV-2 positive sample (C) and one HIV-1/2 antibody negative-HIV-1 antigen positive sample (D). Data was collected and analyzed using DigitalPT; a standardized on-line tool.
Results: Forty-one laboratories returned results. Sample B (HIV negative) was identified by 95% of laboratories (39/41) and samples A and E (HIV-1 positive) by 98% (40/41). No laboratory identified sample C as HIV-2 positive, although 85% (35/41) detected reactivity prompting a referral for further testing. The remaining laboratories identified sample C as HIV-1 positive (4), indeterminate (1) or gave no final status (1). Sample D (HIV antibody negative-antigen positive) was correctly identified by two laboratories as HIV-1 antigen positive while 78% (32/41) detected reactivity, recommending further testing. One laboratory did not provide a final status. Alarmingly, six laboratories called this sample HIV negative.
Conclusion: Although there is a high quality of HIV testing across Canada, introduction of the M53-A guideline would further improve the ability of laboratories to diagnose HIV-1 acute and HIV-2 infection.

1. Introduction

In December 2011, the Clinical and Laboratory Standards Institute (CLSI) published M53-A, *Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus (HIV) Infection; Approved Guideline*.[1] This guideline was a joint effort over two years with expertise from different backgrounds including the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), CLSI and industry.[2] It represents the most significant contribution to HIV testing since the 1989 guideline in which a repeatedly reactive sample by a screening immunoassay, was to be confirmed by a supplemental HIV antibody (Ab) test, usually the Western blot (WB) or the indirect immunofluorescence assay.[3]

Although M53-A contains 6 unique algorithms addressing a range of HIV testing scenarios, Algorithm I (Fig. 1) has generated considerable interest and is likely to have the biggest impact on routine HIV testing. Algorithm I contains three categories of tests; (i) 4th generation HIV Ab and antigen (Ag) combo assay, (ii) HIV-1/HIV-2 discrimination assay and (iii) Nucleic acid testing. One unique feature of this new algorithm is the absence of the HIV-1 WB. Validation data has revealed the shortest HIV diagnostic window period thus far.[4,5]

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; HIV, human immunodeficiency virus; APHL, Association of Public Health Laboratories; CDC, Centers for Disease Control and Prevention; EIA, enzyme immunoassay; WB, Western blot; Ab, antibody; Ag, antigen; NLHRS, National Laboratory for HIV Reference Services; N/T, not tested; Pos, positive; Ind, indeterminate; GS, genetic systems; RIPA, radioimmunoprecipitation assay; LSR, lab-specific report; FDA, Food and Drug Administration; CE, European conformity; Inno-LIA, INNO-LIA HIV I/II score; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

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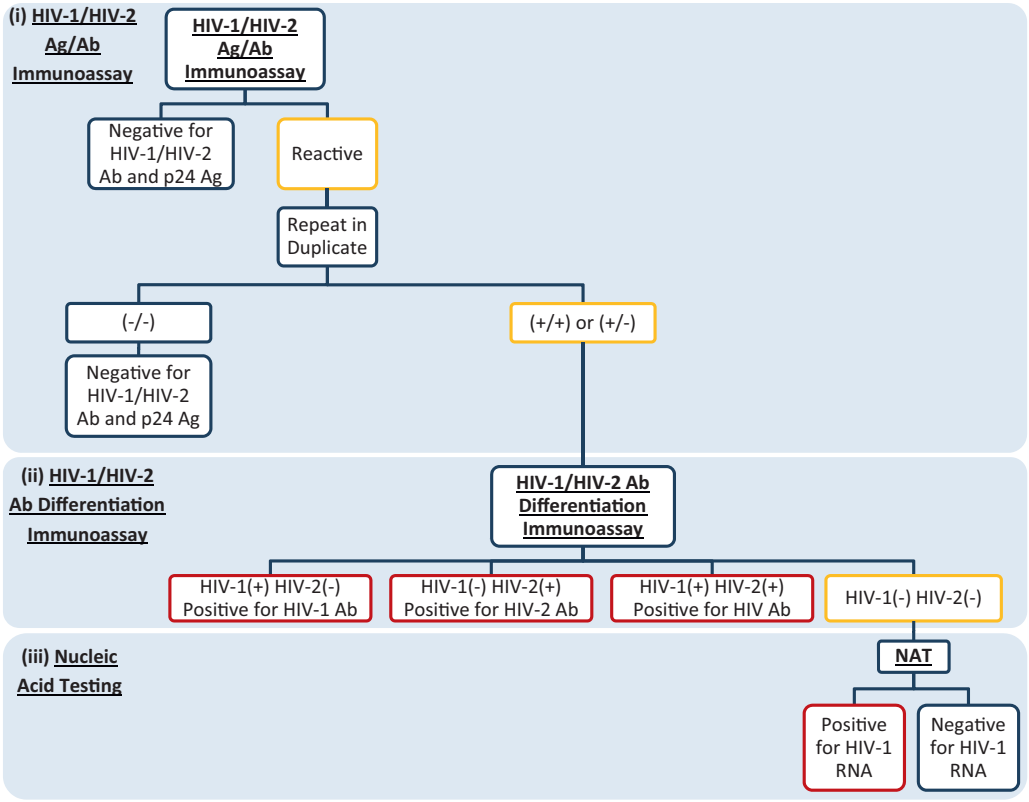

Fig. 1. Clinical and Laboratory Standards Institute M53-A algorithm I.[1]

Panel HIVS425-2012Nov22, sent by the National Laboratory for HIV Reference Services (NLHRS) to forty-two Canadian laboratories was designed to address the impact of M53-A Algorithm I and challenge the wide-ranging tests and algorithms used in Canada.

2. Methods

2.1. Samples

Panel HIVS425-2012Nov22 contained five samples (Table S1); one HIV negative (B) and four HIV positive (A, C, D and E) (Discovery Life Sciences, CA; Seracare Life Sciences, MA) and were extensively characterized (Table S1, Fig. S1). Samples A and E were anti-HIV-1 Ab positive, sample C was anti-HIV-2 Ab positive and sample D was HIV-1/2 Ab negative-HIV-1 Ag positive. Panels were distributed to 42 laboratories under similar conditions to clinical samples.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.04.009.

2.2. Laboratories, testing and data entry

Algorithms and tests range from single screen-only to confirmatory (Table S2, Fig. S2). Panels were shipped November 6th 2012; the closing date for data entry was November 22nd 2012. Results were entered using DigitalPT (Vancouver, BC); an on-line tool for standardized data entry, collation and group analysis. Group data was analyzed by the NLHRS for the final report.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.04.009.

3. Results

Results were returned from 98% of laboratories (41/42) by the deadline to be included in the group analysis. The remaining 41 laboratories' diagnosis for each sample is listed in Table S3.

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3.1. Samples A, B and E

All laboratories correctly identified these three samples. All but two laboratories made an appropriate final serology status or recommendation based on their algorithms.

3.2. Sample C (HIV-2 Ab positive)

Of 41 laboratories, 'none' correctly diagnosed sample C as HIV-2 Ab positive. One laboratory provided no final status or recommendation to refer the sample to a reference or provincial laboratory or request a follow-up sample for further testing. Three laboratories did not provide a final status, only making a recommendation. Although thirty-two laboratories (78%) identified this sample as either HIV-1 Ab positive (4), HIV-1/2 Ab positive (9) or HIV-1/2 Ab/Ag positive (19), a recommendation was made. Five laboratories (12%) made no recommendations; one identified the sample as indeterminate while the other four identified the sample as HIV-1 Ab positive. All five used the HIV-1 WB for confirmatory testing. The algorithm of one of those four laboratories included an HIV-2 EIA in addition to an HIV-1 WB, but they still identified the sample as HIV-1 Ab positive although their HIV-2 screen test was reactive.

3.3. Sample D (HIV-1/2 Ab negative-HIV-1 Ag positive)

Two laboratories (5%) correctly identified this sample as HIV-1 Ag positive but also incorrectly identified it as HIV-1 Ab positive although the confirmatory Ab test was negative. Three laboratories (7%) did not provide a final status and only made a recommendation. Twenty-nine laboratories (71%) reported a wide range of HIV reactivities however they all made recommendations prompting further testing. Six laboratories (15%) identified this sample as HIV-1/2 Ab negative with no further recommendations.

4. Discussion

4.1. What would happen if a laboratory received an HIV-2 sample?

'None' of the Canadian laboratories were able to distinguish sample C as HIV-2 Ab positive. All laboratories, however, reported some level of reactivity with the majority making a recommendation. Four laboratories (10%) reported the sample as HIV-1 Ab positive with no further recommendations.

The majority of HIV screening assays allow for the detection of HIV-1/2 however, there are no approved confirmatory assays for the discrimination of HIV-2 in Canada. Although cross reactivity on the HIV-1 WB often occurs (Fig. S1), it may lead to an 'indeterminate' result or a false diagnosis of HIV-1 positivity. The absence of cross reactivity can occur, resulting in a false-negative diagnosis (internal validation-NLHRS). Even if laboratories referred an HIV-2 Ab sample to a provincial or reference laboratory, it is unlikely these referral laboratories would have had the ability to discriminate HIV-2. In the United States there is currently one FDA approved assay, which discriminates between HIV-1 and HIV-2 (Bio-Rad HIV-1/2 Multispot). The NLHRS has employed the CE-labeled Innogenetics INNO-LIA HIV I/II Score (Inno-LIA) for over 10 years, which can diagnose and discriminate HIV-2. The NLHRS also uses an in-house HIV-2 specific PCR leading to a comprehensive ability to diagnose and discriminate HIV-2 and HIV-1. In Canada, unlicenced assays such as the Inno-LIA must be obtained through the Special Access Program.

4.2. What would happen if a laboratory received an HIV-1 Ab negative-Ag positive sample?

The majority of laboratories that used fourth generation assay detected sample D and made a recommendation. Four laboratories (10%) reported this sample as HIV-1 Ab negative but made a recommendation. These labs used an HIV-1/2 Ab screen test only and it is likely their algorithm takes into consideration that a pre-seroconversion sample, similar to sample D could be missed, prompting further testing.

It is alarming that six laboratories (15%) incorrectly identified Sample D as HIV-1/2 Ab negative with no further recommendations, identifying limitations and weaknesses in their current algorithms to detect *pre*-seroconvertors or acute infection leading to the potential of patients being misdiagnosed as false-negative for HIV. None of these laboratories used a 4th generation (combo) HIV EIA, with most (4/6) still using a 3rd generation HIV-1/2 EIA. The inability to detect these early acute infections and their impact on public health is obvious.

5. Conclusion

The publication of the CLSI M53-A HIV testing guideline is anticipated to have a major impact in industrialized countries. Utilizing the latest technology, it addresses several weaknesses in current testing, including the inability to (i) discriminate HIV-2 and (ii) diagnose acute phase infections (pre-seroconvertors).

There is controversy surrounding this new guideline. A 2012 survey conducted by the APHL revealed cost, workforce/regulatory requirements and remarkably, 'a lack of perceived need' as impediments.[6] While preliminary data demonstrates the cost effectiveness of implementation, the survey revealed replacement of the WB would likely prove challenging.[6,7] The majority of public health laboratories cited the lack of a formal recommendation from the CDC as a major barrier to discontinuing the use of the WB in favor of an HIV-1/HIV-2 discrimination assay.[6] Considering that very little has happened since the original HIV testing guideline in 1989, it is not surprising that end clients, namely clinicians, and laboratorians will need to be educated on new tests and their use in this new algorithm.

Panel HIVS425-2012Nov22 demonstrated deficiencies still exist and improvements need to be made. The M53-A guideline should aid to ensure the highest quality of HIV testing in Canada allowing better diagnosis of HIV-2 infections and early HIV-1 infections.

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Competing interests

None declared.

Ethical approval

Not required.

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